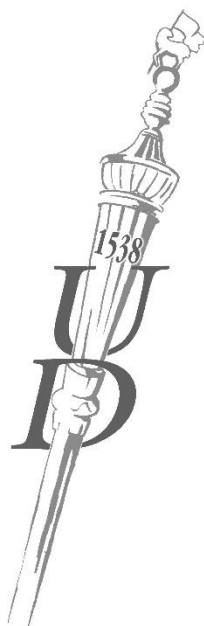


Thesis of the (PhD) Dissertation

**ESTIMATION OF GENETIC VARIABILITY AMONG TRADITIONAL HUNGARIAN
HORSES BY MITOCHONDRIAL DNA ANALYSIS**

Nikolett Sziszkosz

Supervisor: Dr. András Jávora CSc.



UNIVERSITY OF DEBRECEN

Doctoral School of Animal Husbandry

Debrecen, 2017.

TABLE OF CONTENTS

1. BACKGROUND OF THE Ph.D. THESIS	3
2. AIMS OF THE RESEARCH	4
3. METHODS.....	5
3.1 SAMPLE COLLECTION.....	5
3.2 MITOCHONDRIAL DNA TEST	5
4. MAIN OBSERVATIONS OF THE THESIS	9
4.1 MOLECULAR PHYLOGENETIC ANALYSIS OF GIDRAN HORSES	9
4.2 VERIFICATION OF MARE FAMILIES USING MOLECULAR MARKERS	12
4.3 MOLECULAR PHYLOGENETIC ANALYSIS OF HUCUL HORSES	15
4.4 COMPARISON OF HUCUL CYTB AND D-LOOP HAPLOTYPES WITH ADDITIONAL PONY SEQUENCES.....	16
4.5 VERIFICATION OF HORSE MARE FAMILIES USING TWO MOLECULAR MARKERS.....	17
5. NEW AND NOVEL OBSERVATIONS OF THE THESIS.....	20
6. APPLICABILITY OF THE RESULTS	21
7. PUBLICATIONS IN THE TOPIC OF THE THESIS	25

1. BACKGROUND OF THE PhD THESIS

The traditional Hungarian breeds have been developed by professional breeding work and they became a part of our cultural heritage. Consequently, the preservation of their characteristic attributes and their genes as well as the analysis of their genetic structure is important (*Balog, 1997; Mihók, 2016*).

Gidrán has one of the smallest populations among the traditional Hungarian breeds, representing a significant cultural and genetic value in Hungarian livestock breeding (*Mihók, 2006*). Moreover, hucul horses also come from an endangered group, if we consider the few numbers of mares (*Jakabová et al., 2009*). Comprehensive mapping of both breeds' genetic structure is essential for the preservation of their valuable genetic resources. This aspect can be useful for the breed protection plans as well as for understanding their genetic status.

The mitochondrial DNA (mtDNA) is characterized by maternal inheritance and a high degree of polymorphisms. Moreover, it is also suitable for studying the genetic structure of maternal lines (*Taberlet et al., 2011; Jianxing et al., 2012*). Over the past decade, several studies were conducted using the mitochondrial DNA sequences of cytochrome b (*CYTB*) gene or the control region - also known as D-loop - for mapping the origins of domesticated and ancestral horses. The non-coding D-loop region is the most polymorphic part of the mitochondrial genome (*Lopes et al., 2005*). Along with the control region, the examination of the protein coding *CYTB* sequence polymorphisms plays an important role in origin verification, as well as it is useful for phylogenetic studies.

The main aim of my dissertation was to determine the diversity of Gidrán and Hucul mare lines using the aforementioned mtDNA markers. Our further goal was to reveal possible errors or errors that may occur during the management of stud books. Finally, I also investigated the usefulness of an optimized DNA-based method for the discrimination of male families by mtDNA haplotypes.

2. AIMS OF THE RESEARCH

- Mapping and characterizing the genetic structure of Hungarian horses using mitochondrial DNA markers.
- Identification of Gidrán and Hucul mtDNA haplotypes and analysis of the genetic diversity of species by mitochondrial markers.
- Bioinformatical comparison of the genetic features of Hungarian hucul mare populations with selected pony populations from GenBank.
- Comparison of the Gidrán and Hucul mares' genealogical data with the molecular phylogenetic results in order to determine possible discrepancies in the management of traditional studbooks.

3. METHODS

3.1 SAMPLE COLLECTION

Altogether, I analysed 250 hair samples of Gidrán mares originated from different Hungarian breeds: Bakonycsérnye, Balatonalmádi, Biharkeresztes, Budapest, Csákvár, Debrecen, Győr, Gyűrűs, Lulla, Marócpusztá, Nagycsepely, Rédics, Sárkeresztes, Siklós, Tata, Tiszafüred, Zalaszentgrót.

Furthermore, 267 hucul mares were also involved from different Hungarian breeds: Csemő, Gyűrűs, Hárskút, Izsák, Jósavfő (Aggtelek National Park), Kisoroszi, Ólmod, Pécsely, Pénzesgyőr, Pilismaró, Rakamaz, Solt, Szigetcsép, Varbó in our study.

All horses' hair samples included in this study were teared and posted in well-sealed envelopes by the breeder. To avoid contamination, all samples were kept separated from each other at reduced humidity and room temperature until the laboratory utility.

D-loop sequencing was not performed in case of seventeen hucul samples, the available DNA sequence data of the horses were downloaded in FASTA formats from GenBank database instead. The accession number of haplotype sequences determined by *Priskin et al.* (2010) were as follows: KC143336.1- KC143355.1

In the bioinformatics analysis, 22 selected hucul haplotypes were compared with the 35 ponies' *CYTB* and D-loop haplotype sequences which were previously obtained from the NCBI (National Center for Biotechnology Information) database. Since the sequences of two markers were investigated, the analysis was limited to samples with full mtDNA sequences.

3.2 MITOCHONDRIAL DNA TEST

Genomic DNA extraction was made according to the FAO (FAO/IAEA, 2004) instructions. The freely available Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/primer3/>) was used for PCR primer design. Primer pairs were designed to target two polymorphic sites which were previously described as suitable markers for phylogenetic studies: 1192 bp D-loops and 1140 bp long *CYTB* genes for different. Four of the seven primer pairs were specific to the D-loop region, and three primary pairs were specific to the *CYTB*. The specifications of the primers are shown in **Table 1**, while the sequences covered by them are shown in **Figure 1**.

Table 1.: Specifications of primers

Primers		Primer sequences	The length of the products
A	Dloop1_F	ACGACAACAATTTACCCTCA	961 bp
	Dloop1_R	GGGGAAGAAGGGTTGACAGA	
B	Dloop2_F	CCCCCACATAACACCATACC	883 bp
	Dloop2_R	ATCTAGGGGGATGCCTGTCT	
C	Dloop3_F	TCAGCAACCCTCCCAACTAC	652 bp
	Dloop2_R	ATCTAGGGGGATGCCTGTCT	
D	Dloop4_F	ACGTTATTCTCCGCATCAG	350 bp
	Dloop2_R	ATCTAGGGGGATGCCTGTCT	
E	Cyt1_F	TTCCACGTGGAATCTAACC	1092 bp
	Cyt1_R	GTCCGCCGATTTCATGTTAGT	
F	Cyt2_F	GCATTCATGGGCTATGTCCT	643 bp
	Cyt1_R	GTCCGCCGATTTCATGTTAGT	
G	Cyt3_F	TCATCATCACAGCCCTGGTA	556 bp
	Cyt3_R	ATGGTGCTTGCGAGTGGTAT	

```

>gi|577571|emb|X79547.1| Equus caballus mitochondrial DNA sequence, D-loop regio
ATTTCCTCCCTAAACGACAACAATTTACCCTCATGTGCTATGTGCTATCAGATTATAACCCACATAACACCATACCCACCTGACATGCAATAT
CTTATGAATGGCCTATGACGTCGTGCAATAAATTGTCTGCCCATGAATAAAGCATGTACATAATATCATTATCTTACATAAGTACATTATA
TTATTGATCGTGCATACCCCATCCAAGTCAAATCATTCCAGTCAACACGCATATCACAGCCCATGTTCCACGAGCTTAATCACCAGCCGCGGG
AAATCAGCAACCTCCCAACTACGTGTCCCAATCCTCGCTCCGGGCCATCCAAACGTGGGGTTTCTACAATGAAACTATACCTGGCATCTGGT
TCTTCTTCAGGGCCATTCCACCCCAACCTCGGCCATTCTTCCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCATGCTCACAC
ATAACTGTGATTTCATGCAATTTGGTATCTTTTATATTGGGGATGCTATGACTCAGCTATGGCCGTCAAAGGCCCTCAGCCAGTCAATTAATTTG
AAGCTGGACTTAAATTGAACGTTATTCTCCGCAACGCAACCATAAGGTGTTATTTCAGTCCATGGTAGCGGGACATAGGAAACAAGTGCACCT
GTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCT
GTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCTGTGCACCT
CCACATATGTACATTCAACACAATCTTGCCAAACCCCAAAACAAGACTAAACAATGCACAATACTTCATGAAGCTTAACCCCTCGCATGCCAAC
CATAATAACTCAACACACTAACAACTTAAACAGAACTTCCCCCGCCATTAATACCAACATGCTACTTTAATCAATAAAATTTCCATAGACAG
GCATCCCTAGATCTAATTTCTAAAATCTGTCAACCTTCTTCCCCTC

>gi|40806321|gb|AY515159.1| Equus caballus cytochrome B (CYTB) gene
TAATCCACAGTGGAAATCTAACCACGACCAATGACATGAAAAATCATCGTTGATTTCAACTATAAGAACCAATGACAAACATCTGGAAATCTC
ACCCATAATTAAATCATCAATCACTCTTTATTGACCTACCAGCCCTCAAACATTTTCATCATGATGAAACTTCGGCTCCCTCCTAGGAATCT
GCCTAATCCTCCAAATCTTAACAGGCCATTCTAGCCATACACTACACATCAGACACGCAACTGCCTTCTCATCCGCTCACTACATCTGCCGA
GACGTTAACTACGGATGAATTATTGCTACCTCCATGCCAACGGAGCATCAATATTTTTATCTGCTCTTCATTACGTAGGACGCGGCCTCTAC
TACGGCTCTTACACATTCTAGAGACATGAAACATTGGAATCATCTACTTTTACAGTTATAGCTACAACATTCATGGGCTATGTCCTACCATGA
GGCCAAATATCTTTTGGAGGACCAACAGTCAACGAACTCCTATCAGCAATTCCTACATCGGTACTACCTCGTCTGAGTGAATCTGAGTGG
ATTCTCAGTAGACAAAGCCACCTTACCCGATTTTGTCTTCCACTTCATCTACCTTCATCATCACAGCCCTGGTAGTCGTACATTTACTATTT
CTTCACGAAACAGGATCTAATAACCCCTCAGGAATCCCATCCGATATGGACAAAATCCCATCCACCATATTATACAATTAAGACATCTAGG
ACTCTCTCTCTGATCTTGCTCCTACTAACTCTAGTATTATCTCCCCGACCTCCTAGGAGACCCAGACAACCTACACCCAGCTAAACCTCTCAG
CACTCCCCCTCATATTAACCCAGAATGGTCTTCTGCTTTCCTACGCCATCCTACGCTCCATTTCCCAACAACTAGGCGGGCTATTAGCCCTAAT
CCTCTCATCTCTGATCCTCACTCATCCCCACCTCCACATATCAAAACAACGAAGCATAAATTCCGGCCTCTCAGCCAAATGCGTATTCTGACT
CTTAGTGGCAGACTTACTGACACTAATCATGAATCGGGCGGACAGCAGTGGAAACCCCATACGTAATTATCGGCCAAGTGGCCTCAATCTCTACT
TCTCCCTAATCTCATTTTATACCACTCGCAAGCACCATCGAAAACAATCTTCTAAAATGAAGA

```

Figure 1: The *Equine* mtDNA D-loop and *CYTB* sequences in FASTA format. Identical primer pairs are emphasized with the same colours.

After primer design, the most appropriate primer pair was selected from the seven primers by gradient (with multiple temperatures) PCR and the optimal primer adhesion temperature (62.4 °C) was also determined. During the gel electrophoresis, the research was continued with the "E" primer pair that gave the most distinct bands, which covered a longer fragment within the *CYTB* gene:

CYTB gene:

- **14115F** 5'-TTCCCACGTGGAATCTAACC-3'
- **15206R** 5'-GTCCGCCGATTCATGTTAGT-3'

Within the D-loop region I worked with a primer pair covering 298 bp long mtDNA fragment and was previously used by *Priskin et al.* (2010):

D-loop region:

- **15444F** 5'-ACCATCAACACCCAAAGCTG-3'
- **15742R** 5'-GCTGATTTCCTCGCGGCTTGGTG-3' (*Priskin et al.*, 2010)

The primary adhesion temperature was graded at 62.4 °C by the gradient PCR. The amplification was done by PCR using the MJ Research PTC-200 Thermal Cycler (MJ Research, Watertown, MA, USA).

Separation of nucleic acids by gel electrophoresis: 2% Seakemagorose (Lonza, USA), 1X TAE buffer (Thermo Fisher Scientific, Waltham, USA), 0.5 mg/ml Gelred (Biotium, Hayward, CA, USA) for the purpose. The results were checked under UV light.

Purification of the PCR product was carried out using the Viogene DNA / RNA Extraction PCR-M Clean Up System (Viogene-BioTek, Taipei, Taiwan) kit according to the manufacturer's instructions. The quality of the PCR products and DNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer.

Sequencing was performed by Eurofins MWG-Operon (Ebersberg, Germany) and MacroGen Europe (Amsterdam, The Netherlands) with primers also used for PCR.

In the bioinformatic evaluation of the results, the validity of the read nucleotides was evaluated by CodonCode Aligner v. 4.2.7 (*CodonCode Corporation*, 2014). Sequences obtained from sequencing were compared with sequences downloaded from the NCBI database using the

ClustalW algorithm (Larkin *et al.*, 2007). The nucleotide positions were determined based on the reference sequences available in the GenBank database (acc. nr.: X79547) and (acc. nr.: JN398377). Standard diversity values such as polymorphic sites, haplotype and nucleotide diversity were determined using DnaSP5 (Rozas *et al.*, 2009) software. An *Equus asinus* (donkey) (acc. nr.: NC001788) sequence was used as an outgroup in case of both markers.

Previously unpublished haplogroups in the NCBI database were identified using the BLAST (Basic Local Alignment Search Tool) online match search engine (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The DnETree (<http://www.dnctree.org/>) haplotype analysis was performed in case of both breeds. Phylogenetic trees were visualized by the DnETree for Windows software (<http://dnctree.cirad.fr/>). The nucleotide positions of the DnETree haplotype are based on the reference sequence (GenBank acc. nr.: JN398377).

The visualization of the mtDNA haplotypes of mare families and the genetic conditions of different pony sequences was visualised by the Network 4.6.0.0. (Bandelt *et al.*, 1999) and PopArt (<http://popart.otago.ac.nz>) softwares using median-joining network method.

Phylogenetic analyses were performed using the MEGA6 (Tamura *et al.*, 2013) software packages. The maximum likelihood algorithm was used for drawing phylogenetic trees (Hasegawa *et al.*, 1985), moreover bootstrap analysis with 1000 values was also performed (Podani, 2003).

The most suitable nucleotide substitution model for the sequences was selected by the jModelTest (Posada, 2008) software. Accordingly, I used the Hasegawa-Kishino-Yano (HKY) plus gamma model for *CYTB* (Hasegawa *et al.*, 1985) and the Tamura 3-parameter (T92) plus gamma models for the D-loop marker in case of both breeds.

4. MAIN OBSERVATIONS OF THE THESIS

4.1 MOLECULAR PHYLOGENETIC ANALYSIS OF GIDRAN HORSES

In this study, 250 and 246 Gidrán samples evaluated in case of *CYTB* and D-loop markers, respectively. Following the DNA sequencing and bioinformatics normalization of the raw data, altogether 686 nucleotides within the *CYTB* gene and 202 nucleotides within the D-loop region were considered for further studies. Diversity indicators are summarized in **Table 2**.

Table 2: The diversity values of the mtDNA markers in Gidrán

mtDNA markers	Nr. of nucleotides	n	Nr. of haplotypes	Polymorphic sites	Haplotype diversity \pm SD	Nucleotide diversity \pm SD
<i>CYTB</i>	686	250	24	23	0,874 \pm 0,011	0,005 \pm 0,001
D-loop	202	246	32	26	0,914 \pm 0,008	0,021 \pm 0,001
Merged*	893	242	49	-	0,940 \pm 0,006	0,008 \pm 0,001

**CYTB* and D-loop markers were combined for the analysis

The analysis of the sequences both mtDNA marker analyses confirmed the significant genetic variability of Gidrán horses. Twenty-three polymorphic sites were found in *CYTB* (twenty-one SNPs, and two insertion/deletion), representing 3.35% polymorphism considering the full length (686 bp) sequence. The D-loop marker exhibited somewhat more 26 variable sites, representing 12.9% of the total 202 bp sequence. Among polymorphic nucleotides, besides the 25 SNPs, only one insertion/deletion was identified. Both marker sequences were rich in adenine (A) / thymine (T), representing 55% for *CYTB* markers and 64.1% for D-loop.

Both markers showed high haplotype diversity values: 0.8735 ± 0.011 for *CYTB* and 0.9136 ± 0.008 for *D-loop*. The nucleotide diversity values were also high: 0.00472 ± 0.00017 (*CYTB*) and 0.02091 ± 0.00068 (D-loop). The paired genetic distances between haplotypes were 0.001-0.013 (*CYTB*) and 0.005-0.063 (D-loops). According to the phylogenetic literature, it can be stated that in contrast to other species (Ali *et al.*, 2015) studying the *CYTB* marker is relatively rare in horses (Li, *et al.*, 2006, Jianxing *et al.* The observed *CYTB* nucleotide diversity parameters are similar to the data of Chinese domestic horses. Yues *et al.* (2012) described 114 different haplotypes by studying the 323 Chinese domestic horses and 84 selected sequences from the GenBank database

(Yue *et al.*, 2012). The nucleotide diversity value is between 0.005 and 0.002. In addition, haplotype diversity ranges from 0.706 to 0.975, which also overlaps with my results (Gidrán: 0.874; Hucul: 0.835) (Yue *et al.*, 2012). Qin *et al.* (2009) identified twenty-two Lichuan horses using 1140 bp length *CYTB* sequences. Similarly, to the results described above, high haplotype and nucleotide diversity values (0.840 and 0.048) were observed. The observed variability of *CYTB* in my study also highlights the genetic diversity of Gidrán, however the observed haplotype numbers and nucleotide diversity were lower compared to the D-loop marker.

Taking into account the number of D-loop haplotypes, similar results have been already described in Lusitan (27 haplotypes / 145 horses) (Lopes *et al.*, 2005), Lipizzan (37 haplotypes / 212 horses) (Kavar *et al.*, 2002), Arabian (27 haplotypes / 200 horses) (Bowling *et al.*, 2000) horses, and higher numbers in case of Kiso horses (7 haplotypes / 136 horses) (Takasu *et al.*, 2014). The observed haplotype and nucleotide diversity values were also consistent with previous research results. Recent results are roughly similar to the Iranian horse population's data (0.02) (Moridi *et al.*, 2013). Considering the relatively high values, the involved Hungarian horse populations are genetically more variable than the Kerry bog (0.0155 ± 0.0040), the Sulphur (mustard) mustang (0.001 ± 0.002). On the other hand, the genetic structure of Marwari (0.03973 ± 0.01262) or Sorraia (0.104 ± 0.012) is even more diverse than the Gidrán or Hucul (Luís *et al.*, 2006; Prystupa *et al.*, 2012; Devi & Ghosh, 2013). Sequences of the haplotypes were uploaded to the GenBank database with the following identifiers: KT792934 - KT792957 and KT818891 - KT818922.

All phylogenetic analyses regarding both markers were performed separately and in a combination of the two sequences. Of the 31 Gidrán mare families, I successfully isolated 24 different haplotypes using *CYTB* marker, and 32 different haplotypes using the D-loop marker. Similarly, diverse data were described in a previous Hucul study (Kusza *et al.*, 2013) as well as in Zemaitukan horses, even though both varieties had a heavy bottleneck effect during their history (Cothran *et al.*, 2005; Kusza *et al.*, 2013). The observed high D-loop and *CYTB* haplotype diversity confirms the fact that Gidrán originates from several maternal lines, which is supported by the fact that the recently known male families are derived from 16 founding mares (Mihók, 2006).

Two different reference sequences were used in the phylogenetic analysis of the Gidrán horses: X79547 and JN398377. Most phylogenetic studies use only the X79547 sequence published in 2005, which makes it possible to classify D-loop haplotypes into haplotypes as defined by Jansen *et al.* (2002) and Achilli *et al.* (2012). On the other hand, novel studies, such as the

DomeTree database, use the JN398377 GenBank reference sequence for phylogenetic studies. This latter sequence was realized in 2012 and it is more accurate than the previous X79547 sequence.

Two haplotypes (Ht1_{CYTB} and Ht1_{D-loop}) of the Gidrán haplotypes was identical to the reference sequence, and haplotypes differed in maximum of 6 (*CYTB*) and 9 (D-loop) nucleotides.

Of the 24 *CYTB* haplotypes Ht1_{CYTB} (n=54), Ht2_{CYTB} (n=49) and Ht6_{CYTB} (n=44) were the most common haplotypes. Seven haplotypes, however, represented only one to one individual Ht11_{CYTB}, Ht17_{CYTB}, Ht18_{CYTB}, Ht20_{CYTB}, Ht21_{CYTB}, Ht22_{CYTB}, and Ht24_{CYTB}. The phylogenetic "maximum likelihood" tree was represented by the haplotype type 250 of the 250 Gidrán mare.

The most common haplotypes for the D-loop marker were Ht6_{D-loop} (n=47), Ht16_{D-loop} (n=35) and Ht1_{D-loop} (n=25). Ten haplotypes, however, were found in only one animal: Ht14_{D-loop}, Ht17_{D-loop}, Ht18_{D-loop}, Ht21_{D-loop}, Ht24_{D-loop}, Ht26_{D-loop}, Ht28_{D-loop}, Ht29_{D-loop}, Ht30_{D-loop}, and Ht31_{D-loop}.

The performed Basic Local Alignment Search Tool (BLAST) analysis among NCBI sequences revealed six new *CYTB* (Ht5_{CYTB}, Ht8_{CYTB}, Ht11_{CYTB}, Ht14_{CYTB}, Ht20_{CYTB}, and Ht21_{CYTB}) and four D-loop (Ht12_{D-loop}, Ht28_{D-loop}, Ht29_{D-loop}, and Ht32_{D-loop}) haplotypes.

All haplotypes were also classified into haplogroups. The 32 D-loop haplotypes, defined by *Jansen et al.* (2002), can be classified into seven main D-loop haplotypes with a distribution of: A: 31%, B: 3%, C: 28%, D: 19%, E: 3%, F: 13%, and G: 3%. Considering the Achilli's D-loop classification which contains 18 haploid groups, only the haplogroup E did not represent itself in the examined Gidrán mare population.

According to the DomeTree data (*Peng et al.*, 2015), the *CYTB* haplotypes were classified into ten (A1b, AQ, B1a2, D, G1a, G1b, G3, H, HQ and MN), while D-loop haplotypes were classified into eighteen (A1d, A3, AB, B1, B1a, D, G, G1, G1a, H1, I, J1, J2, K, L, O, OP, R) haplogroups. Unlike other haplogroup classifications, the DomeTree database is based on a full mtDNA sequence and not only on the D-loop sequences. From this point of view, I also classified the samples based on the two markers. In this case nine haplogroups have been obtained (A1, AB, H, H-I, J-K, M-N, M-Q, B1, B1a).

4.2 VERIFICATION OF MARE FAMILIES USING MOLECULAR MARKERS

The practical usage of mtDNA markers was also analysed for the identification of possible errors / misspellings in the studbooks as well as the investigation of the efficacy of mtDNA analysis in the classification of mare families by haplotypes.

In the first step, phylogenetic trees were prepared separately for each marker. Of the thirty-one mare families, 15 (48.4%) haplotypes were separated by *CYTB* and 17 (54.8%) haplotypes were separated by D-loop marker. The borodi 14 and 18, as well as mezőhegyesi 1 families could be separated by only the usage of *CYTB*, while the family of 2, 3, 19 and borodi 2 families could be separated by only the usage of the D-loop markers.

It is important to mention, that some families were not necessarily separated into a distinct haplotype, or multiple mare families share a haplotype. Consequently, the combined use of *CYTB* and D-loop markers (*Pedrosa et al.*, 2005) could be a possible solution for the more accurate association of mare family and mtDNA haplotypes. In the next phase of my work, I also performed all analysis with the combining markers. In the case of the combined analysis, I found the network representation as the most suitable visualization for the haplotypes of mare families due to the large number of individuals (Figure 2). Based on this observation circular trees were not created for hucul horses.

In line with our expectations, using *CYTB* and D-loop, I gained more accurate results, because seven additional Gidrán families (mezőhegyesi 7, 8 and 21, borodi 5, 17, and 19, népies 23) were also separated. With the combination of the two markers, borodi 1 and borodi 7 formed a common haplotype. It is important to mention that none of the markers could separate five mare families (mezőhegyesi 5, 11, 13, 14 and borodi 18).

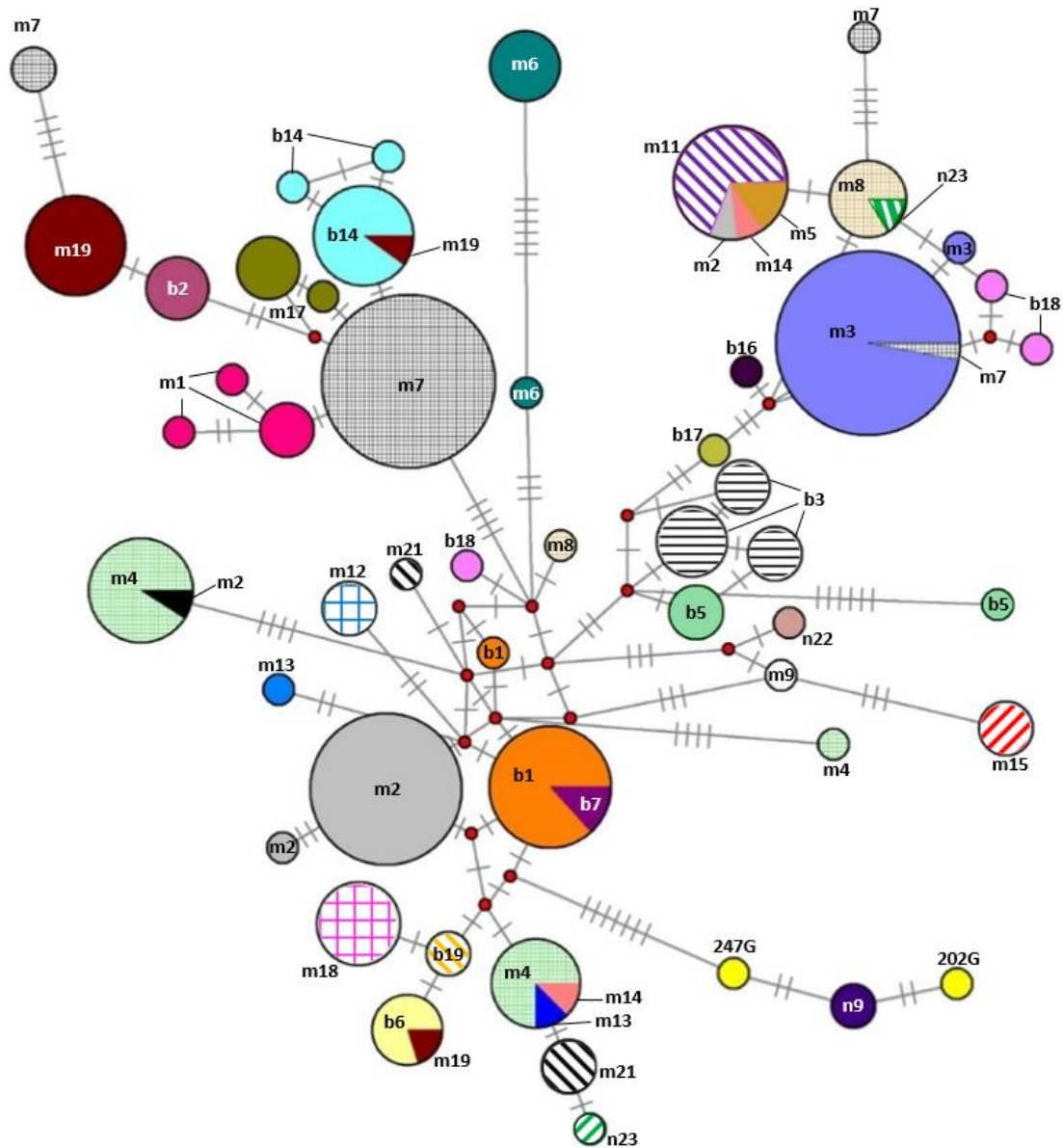


Figure 2: A median-joining phylogenetic network (Bandelt *et al.*, 1999) comprising a total of 242 Gidrán sample sequences using merged *CYTB* and D-loop markers. The mare families were unknown not known for yellow-labelled 247G and 202G samples. The abbreviation of mare family names in the figure are the following: m = mezőhegyesi; b = borodi; n = népies.

Altogether the mtDNA polymorphisms based studbook classification were performed in 247 Gidrán mare samples. As a result of the molecular study, the matching of haplotypes to the mare family indicated in the studbook were succeeded in altogether 227 (92%) individuals.

Considering the total number of horses in recent study, nine individuals (3.6%) fell into a haplotype that is typical of other mare families indicating misspelling in the studbook management. This rate is excellent in comparison to the stud books of Lipica and Polish horses, where the discrepancy was around 11-11% (*Kavar et al.*, 2002). The differences in the case of two animals affected the mezőhegyesi 2, 14 and 19, and in one case by mezőhegyesi 7, 13 and the népies 23 family.

Almost 1.6% of the samples (of different mare families) belonged to the same haplogroup which can be explained by several reasons: 1.) misspellings in the studbook, 2.) common origin of the horses, or 3.) the low-rate polymorphisms in the examined markers. Consequently, two-two mare families formed a common haplotype (borodi 1 and 7 and mezőhegyesi 5 and 11). Horses from the borodi 7 family did not form a separate haplotype, as did the members of the mezőhegyesi 5 family. The mares of borodi 7 family shared a haplotype with borodi 1, while mares of mezőhegyesi 5 shared a haplotype with the members of mezőhegyesi 11 family. These differences are in line with the data on the Gidrán breeds' history (*Mihók*, 2006).

A total of 32 haplotypes were isolated by the D-loop marker, so we could map mare families that could not be isolated by the single *CYTB*. These were mezőhegyesi 2, 3, and 19, as well as borodi 2. On the other hand, the *CYTB* sequences were more informative for the examination of borodi 14, borodi 18, the mezőhegyesi 1 families (Figure 2). The significance of the combined use of the two mtDNA markers is emphasized by the fact that the separation of eight Gidrán mare families were possible only by the merged markers, moreover the members of the borodi 19 family were only distinguished by this approach as well.

Interestingly, the members of mezőhegyesi 4 mare family form two distinct haplotypes using both markers. Furthermore, horses of this family formed two separated groups with merged markers. This observation indicates mezőhegyesi 4 mare family can be divided into two molecular sub-families. This phenomenon can be explained by the fact that this mare family had been divided into two families during the time (*Mihók*, 2006). The mare families were unknown of three horses, but they also formed separated haplotypes.

4.3 MOLECULAR PHYLOGENETIC ANALYSIS OF HUCUL HORSES

Among the collected hucul samples, 265 samples were assessed for the *CYTB* marker and 267 samples for the D-loop region. The diversity values of the Hucul are summarized in **Table 3**. Along with the values of the two mtDNA markers – similar to the Gidrán horses – results of combined marker sequences were also presented here.

Table 3: The diversity values of the mtDNA markers in Hucul

mtDNA markers	Number of nucleotides	n	Nr. of haplotypes	Polymorphic sites	Haplotype diversity \pm SD	Nucleotide diversity \pm SD
<i>CYTB</i>	686	265	13	13	0,835 \pm 0,009	0,005 \pm 0,001
D-loop	202	267	22	21	0,878 \pm 0,009	0,024 \pm 0,001
Merged*	893	240	30	-	0,889 \pm 0,01	0,009 \pm 0,001

**CYTB* and D-loop markers were combined for the analysis

Compared to the Gidrán breed, the genetic structure of hucul horses has been analysed in earlier publications. These studies are mainly based on polymorphisms of D-loop sequences or microsatellite examinations (*Kusza et al.*, 2013; *Priskin et al.*, 2010; *Georgescu et al.*, 2011). The significance of the *CYTB* marker analysis has not yet been investigated in Hucul horses. Using the *CYTB* marker, altogether 13 variable positions were identified (all nucleotide changes were SNPs). Considering the 686 bp length of the sequence, this number indicates 1.9% polymorphism. This value is considerably smaller than Gidrán's *CYTB* polymorphism percentages, which implies that *CYTB* may be less informative about the Hucul diversity. On the other hand, the D-loop was more polymorphic than *CYTB*. The 202 bp long D-loop marker contained 22 polymorphic sites, covering 10.9% of the total sequence, which is close to the ratio found in Gidrán, and only one insertion / deletion (indel) was identified among polymorphic nucleotides.

Similarly to Gidrán, high haplotype diversity was also observed in hucul horses: 0.835 ± 0.009 for *CYTB* and 0.878 ± 0.009 for D-loop. In addition, the nucleotide diversity values were also high: 0.00484 ± 0.00013 (*CYTB*) and 0.02356 ± 0.00055 (D-loop). The genetic distance between the haplotypes in case of *CYTB* was between 0.001 and 0.013, while in case of the D-loop the distance was between 0.005 and 0.063. Previous studies also report a similar variability in the genetic structure of Hucul, however the Hucul has been affected by two known bottleneck effects (*Kusza et al.*, 2013; *Priskin et al.*, 2010)

Of the 31 Hucul mare families, 13 formed distinct haplotypes by the *CYTB* marker, while 22 formed distinct haplotypes by D-loop. Of the thirteen *CYTB* haplotypes Ht1_{*CYTB*} (n = 58), Ht10_{*CYTB*} (n = 57) and H2_{*CYTB*} (n = 56) were the most frequent, while Ht4_{*CYTB*}, Ht8_{*CYTB*}, Ht12_{*CYTB*} haplotypes were identified for only one-one individual. The relationship between the 13 haplotypes of *CYTB* was visualized on a "maximum likelihood" phylogenetic tree.

The most frequent D-loop haplotypes were Ht5_{D-loop} (n=62), Ht4_{D-loop} (n=38), Ht3_{D-loop} (n=37), Ht6_{D-loop} (n=36), and nine haplotypes Ht12_{D-loop}, Ht14_{D-loop}, Ht15_{D-loop}, Ht16_{D-loop}, Ht17_{D-loop}, Ht18_{D-loop}, Ht19_{D-loop}, Ht20_{D-loop}, Ht21_{D-loop}, Ht22_{D-loop} were unique to one and one animal.

The D-loop haplotype 22 can be classified into five main D-loop haplotypes: A: 45%, C: 23%, D: 14%, F: 14%, and G: 5%. Haplogroup A was dominant in Hucul horses, similar to the results of the study by *Kusza et al.* (2013). According to *Jansen et al.* (2002) the majority of the Przewalski horses belong to the "A" haplogroup. Interestingly, Przewalski horses are one of the closest relatives of Huculs, so the obtained results further strengthen this relationship between them.

Regarding to the D-loop haplogroups described by *Achilli et al.* (2012), Hucul mares could be classified into nine haplogroups (A1; A'B'C'D'; E'F'G'; I; L; M; N; O; R). Based on DnTSP classification, I observed altogether 15 (A, A1b, A2, A-I, B1b, E-G, F-G, G, L, L2, L3a2, M-Q, Q2a, R) different haplogroups.

4.4 COMPARISON OF HUCUL CYTB AND D-LOOP HAPLOTYPES WITH ADDITIONAL PONY SEQUENCES

The hucul haplotypes obtained during our work were compared with additional ponies' sequences of different geographic areas by bioinformatics analysis. The main purpose of these investigations was to analyse the genetic diversity among ponies (including Hucul) from different geographic areas. For more reliable results, a total of 35 samples were selected where the *CYTB* and the D-loop sequences were also available.

Based on our results, the genetic structure of the Hungarian Hucul horses is more diverse, compared to the data of other ponies. Six Hucul *CYTB* haplotypes formed common haplotypes with Yakut, Shetland, Przewalskii, Mongolian, Caspian, Chincoteague, Exmoor, Konik, Noriker, Fjord, Icelandic and German riding ponies (except Debaos and Welsh ponies). Regarding D-loop, eight haplotypes of the Hucul haplotypes formed a common haplotype with Yakut, Mongolian, Caspian,

Welsh, Chincoteague, Exmoor, Conic, German Riding Pony, and in 14 cases formed separated group. Based on the results obtained, mtDNA markers because of their polymorphic features are useful for mapping the genetic structure within the given species, as well as for isolation mare families. On the other hand, they are not suitable for separating animals according to the geographical habitat.

4.5 VERIFICATION OF HORSE MARE FAMILIES USING TWO MOLECULAR MARKERS

A comparative analysis of mtDNA haplotypes and mare families was performed in a total of 240 mares involved the D-loop sequences of 19 Hucul mares from a study by *Priskin et al.* (2010).

The mtDNA analysis showed clearly identical results with studbook registry in case of 187 (78%) horses. Aspiráns and Árvácska families from Aggtelek represent the two most frequent mare families among the Hungarian Hucul mare families. Moreover, mares of the 12 Sarata and 4 Kitca originated from Lucina are also common. Based on the molecular results, these individuals formed well-separated, mare-family-specific haplotypes. It is important to point out that the members of Apiráns family formed same haplotypes.

Incomplete studbook classification can happen in case of 30 mares (12.5%). In these cases, the individuals belonging to the above-mentioned families did not create a common haplotype using mtDNA markers. Due to the large number of cases, it is not surprising that most misspellings happened in the following families: Árvácska (6), 4 Kitca (5), 12 Sarata (5). During the comparison, I found three errors in the 882 Gelnica family, which is originated from the formally Slovakian breeds. In addition, the possibility of errors was also found in the studbook classification of Wrona, 5 Plosca, 3 Tatarca, 2 Lucina, 17 Aglaia, 11 Rotunda and 1 Panca families. Mare families with such a large number of individuals should be subjected to greater selection pressure to maintain the genetic balance.

In twenty-four cases (10%), multiple mare families belonged to a single haplotype. In eight cases, the individuals of the 5 Plosca family had a common haplotype with Aspiráns horses. These results may suggest common origins of the two families.

Lower selection pressure should be subjected to families with smaller sample size. Seven mares formed a common haplotype with the 3 Tatarca, 70 Sekacka and Wydra families. I case of

nine horses, 4 Kitca and 17 Aglaia shared a common haplotype. Due to the diminished number of individuals, the position of 3 Tatarca family is endangered among Hucul mare families. Based on our results, members of the 3 Tatarca family did not create a specific haplotype, but shared a common haplotype with the Wydra and the Sekacka horses. It is important to note, that studbook classification of four horses may be registry errors. In case of two members of Árvácska family formed a common haplotype with 4 Kitca and 17 Aglaia horses based on the molecular study. Similarly, horses of 2 Lucina and the 882 Gelnica families also shared a common haplotype with 4 Kitca and 17 Aglaia horses. Increase of the number of these mare families in Hungary is also important, but it is not impossible because there is an adequate number of individuals in Slovakia and Poland (*Mihók*, 2016). The mtDNS results also confirmed that the members of Wydra and Sekacka families besides 3 Tatarca also deserve a special attention.

In the study, mare families were unknown in the case of eight mares (3%). Unique haplotypes were observed in the case of two mares (5 Plosca and Wrona). Even though there were only one sample available from four (825 Agla, 84 Polonia, 90 Macocha, Bajkalka) families, each of the four families formed a separate haplotype.

The comparative analysis of mtDNA haplotypes and the studbook data was also performed in case 19 Hucul samples previously published by *Priskin et al.* (2010), which also represented the infrequent mare families like 5 Plosca, 825 Agla, 84 Polonia, Bajkalka, Bukovina, Wolga. The aim of this study was to test the suitability and effectiveness of combined (*CYTB* - D - loop) markers.

Thirteen individuals (68%) with our combined marker haplotypes were similar to the result of *Priskin et al.* (2010). The only individual in 84 Polonia family formed a unique haplotype using combined markers.

For four samples, the use of combined markers proved to be more effective in associating mare families with mtDNA haplotypes. Two horses belonging to the Bucovina family formed a separated haplotype. In addition, each horse of the 825 Agla and Bajkalka families also formed a separated haplotype. These horses in Priskin's study formed a common "825 Agla/Lucina/Bukovina" haplotypes using the single D-loop marker. The accuracy of the combined analysis emphasizes that 2 Lucina horses did not overlap with these samples.

While in Priskin's study the "*Hroby Bolyhos*" belonged to the 5 Plosca family formed a distinct haplotype, based on my results, it shared a haplotype with Aspiráns horses.

5. NEW AND NOVEL OBSERVATIONS OF THE THESIS

1. In my dissertation, the genetic structure of Gidrán's and Hucul's maternal lines was analysed. Based on the best of our knowledge, my study was the first mtDNA based analysis in Gidrán horses. Furthermore, I was the first to describe the sequence polymorphisms of *CYTB* gene, as well as the first to identify its haplotypes in Hucul horses. I separated 24 Gidrán and 13 Hucul haplotypes according to the polymorphisms of *CYTB*, and 32 Gidrán and 22 Hucul haplotypes according to the D-loop polymorphisms. In this study, I identified six novel *CYTB* (Ht5_{*CYTB*}, Ht8_{*CYTB*}, Ht11_{*CYTB*}, Ht14_{*CYTB*}, Ht20_{*CYTB*}, Ht21_{*CYTB*}) and for D-loop (Ht12_{D-loop}, Ht28_{D-loop}, Ht29_{D-loop}, Ht32_{D-loop}) haplotypes of Gidrán horses, and two new D-loop (Ht16_{D-loop}, Ht18_{D-loop}) haplotypes in Hucul horses. Sequences of both *CYTB* and *D-loop* haplotypes were uploaded to the freely available GenBank database.
2. I described, that the Hungarian Gidrán and Hucul traditional Hungarian horses assess a high grade of genetic diversity. It is also supported by the observed high diversity values. The haplotype diversity value was 0,874 (Gidrán) and 0,835 (Hucul), as well as 0,914 (Gidrán) and 0,878 (Hucul) regarding the *CYTB* and D-loop markers, restrictively.
3. A comparative analysis between Hucul and other ponies from different geographic localisation revealed, that both *CYTB* and D-loop are appropriate markers for mapping genetic heterogeneity of a given breed, but they are not suitable for the separation of haplotypes among different geographical areas.
4. I confirmed, that the management of studbooks is rather adequate. I found that based on the mtDNA haplotypes 91,9% of Gidrán mares and 78% of Hucul horses showed associations with the studbook data.
5. I established, that the combined *CYTB* and D-loop mtDNA markers allow a more adequate comparison of the molecular data with studbook registry. These markers can be used for mapping the genetic structure of other traditional horse breeds, as well as for the genetic based verification of studbooks.

6. APPLICABILITY OF THE RESULTS

1. In my dissertation, I analysed the diversity of mare families of Gidrán and Hucul horses using mtDNA markers. I made the sequences of the haplotypes available in the GenBank database. I demonstrated, that both breeds assess mare family specific mtDNA haplotypes.
2. One novelty and the result of my research, that the combination of *CYTB* and D-loop markers efficiently applied to the comparison of studbook data with mtDNA haplotypes.
3. The results of mtDNA analysis, as well as the observed misspellings in the studbooks could be useful in breeding programs of the National Kisbéri-félvér and Gidrán Breeders Association and the National Pony and Small Horse Breeders Association.
4. In my dissertation, I highlighted the importance of the molecular marker based classification of horses. However, studbook's data are mostly adequate, mtDNA markers could be used for the classification of horses in question into mare families.
5. My results could be also useful for the preservation plans of other breeds, allowing the identification of the genetic status of the species and maintain the rare haplotypes.

7. REFERENCES

1. Achilli, A. - Olivieri, A. - Soares, P. - Lancioni, H. - Kashani, B. H. - Perego, U. A. - Nergadze, S. G. - Carossa, V. - Santagostino, M. - Capomaccio, S. (2012): Mitochondrial genomes from modern horses reveal the major haplogroups that underwent domestication. *Proceedings of the National Academy of Sciences* 109. 2449-2454.
2. Ali, M. E. - Razzak, M. A. - Hamid, S. B. A. - Rahman, M. M. - Al Amin, M. - Rashid, N. R. A. (2015): Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. *Food Chemistry* 177. 214-224.
3. Balog, Á. (1997): A biodiverzitás megőrzése. Budapest OMIKK.
4. Bandelt, H.J. - Forster, P. - Röhl, A. (1999): Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.
5. Bowling, A. T. - Del Valle, A. - Bowling, M. (2000): A pedigree-based study of mitochondrial d-loop DNA sequence variation among Arabian horses. *Animal Genetics* 31. 1-7.
6. Cothran, E. - Juras, R. - Macijauskiene, V. (2005): Mitochondrial DNA D-loop sequence variation among 5 maternal lines of the Zemaitukai horse breed. *Genetics and Molecular Biology* 28. 677-681.
7. Devi, K. M. - Ghosh, S. K. (2013): Molecular phylogeny of Indian horse breeds with special reference to Manipuri pony based on mitochondrial D-loop. *Molecular Biology Reports* 40. 5861-5867.
8. Hasegawa, M. - Kishino, H. - Yano, T. A. (1985): Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22. 160-174.
9. Jakabová, D. - Gralak, B. - Buróczyová, M. - Buleca Jr, J. - Maróti-Agóts, Á. - Massányi, P. - Zöldág, L. (2009): Diversity analysis of Hucul horse population based on molecular genetic data. *Magyar Állatorvosok Lapja* 131. 685-691.

10. *Jianxing, C. - Yujiang, S. - Qingjie, P. - Lingjiang, M. - Lai, M.* (2012): Analyses of Differences in the Maternal Structure of Different Geographical Domestic Horses. Chinese Agricultural Science Bulletin 14. 013.
11. *Kavar, T. - Brem, G. - Habe, F. - Sölkner, J. - Dovč, P.* (2002): History of Lipizzan horse maternal lines as revealed by mtDNA analysis. Genetics Selection Evolution 34. 635-648.
12. *Kusza, S. - Priskin, K. - Ivankovic, A. - Jedrzejewska, B. - Podgorski, T. - Jávör, A. - Mihók, S.* (2013): Genetic characterization and population bottleneck in the Hucul horse based on microsatellite and mitochondrial data. Biological Journal of the Linnean Society 109. 54-65.
13. *Larkin, M. A. - Blackshields, G. - Brown, N. - Chenna, R. - Mcgettigan, P. A. - Mcwilliam, H. - Valentin, F. - Wallace, I. M. - Wilm, A. - Lopez, R.* (2007): Clustal W and Clustal X version 2.0. Bioinformatics 23. 2947-2948.
14. *Li, J.-L. - Shi, Y.-F. - Bu, R.-Q. - Mang, L.* (2006): PCR-RFLP analysis of the mtDNA Cytb gene in three different horse breeds. Yi chuan 28. 933-938.
15. *Lopes, M. S. - Mendonça, D. - Cymbron, T. - Valera, M. - Da Costa-Ferreira, J. - Da Câmara Machado, A.* (2005): The Lusitano horse maternal lineage based on mitochondrial D-loop sequence variation. Animal Genetics 36. 196-202.
16. *Luís, C. - Bastos-Silveira, C. - Cothran, E. G. - Do Mar Oom, M.* (2006): Iberian origins of New World horse breeds. Journal of Heredity 97. 107-113.
17. *Mihók, S.* (2012). Gidrán Méneskönyv. Budapest, Kisbéri és gidrán lótenyésztő országos egyesület.
18. *Mihók, S.* (2006). A gidrán ló monográfiája. Debrecen, Kisbéri és Gidrán Lótenyésztő Országos Egyesület.
19. *Mihók, S.* (2016). Fajtafenntartás, génmegőrzés, lóhasználat (változás) Debrecen, Póni- és Kislótenyésztők Országos Egyesülete
20. *Moridi, M. - Masoudi, A. - Vaez Torshizi, R. - Hill, E.* (2013): Mitochondrial DNA D-loop sequence variation in maternal lineages of Iranian native horses. Animal Genetics 44. 209-213.

21. *Peng, M. S. - Fan, L. - Shi, N. N. - Ning, T. - Yao, Y. G. - Murphy, R. W. - Wang, W. Z. - Zhang, Y. P. (2015): DomeTree: a canonical toolkit for mitochondrial DNA analyses in domesticated animals. Molecular Ecology Resources 15. 1238-1242.*
22. *Posada, D. (2008): jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25. 1253-1256.*
23. *Prystupa, J. M. - Hind, P. - Cothran, E. G. - Plante, Y. (2012a): Maternal lineages in native Canadian equine populations and their relationship to the Nordic and Mountain and Moorland pony breeds. Journal of Heredity 103. 380-390.*
24. *Qin, F. - Wang, X.-B. - Zhang, Y.-S. - Zhang, M.-L. - Li, X.-F. - Lei, C.-Z. (2009): Analyses on mtDNA cytb genetic diversity in Lichuan horse. Hubei Agricultural Sciences 12. 11-12.*
25. *Rozas, J. - Librado, P. - Sánchez-Delbarrio, J. - Messeguer, X. - Rozas, R. (2009): DnaSP v5. DNA 9. 16.*
26. *Taberlet, P. - Coissac, E. - Pansu, J. - Pompanon, F. (2011): Conservation genetics of cattle, sheep, and goats. Comptes Rendus Biologies 334. 247-254.*
27. *Takasu, M. - Ishihara, N. - Tozaki, T. - Kakoi, H. - Maeda, M. - Mukoyama, H. (2014): Genetic Diversity of Maternal Lineage in the Endangered Kiso Horse Based on Polymorphism of the Mitochondrial DNA D-Loop Region. The Journal of Veterinary Medical Science 76. 1451-1456*
28. *Tamura, K. - Stecher, G. - Peterson, D. - Filipski, A. - Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30. 2725-2729.*
29. *Yue, X. P. - Qin, F. - Campana, M. G. - Liu, D. H. - Mao, C. C. - Wang, X. B. - Lan, X. Y. - Chen, H. - Lei, C. Z. (2012): Characterization of cytochrome b diversity in Chinese domestic horses. Animal Genetics 43. 624-626.*

8. PUBLICATIONS IN THE TOPIC OF THE THESIS



UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY



Registry number: DEENK/259/2017.PL
Subject: PhD Publikációs Lista

Candidate: Nikolett Sziszkosz
Neptun ID: KL9K79
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10050429

List of publications related to the dissertation

Hungarian book chapters (1)

1. **Sziszkosz, N.**, Kusza, S., Jávör, A., Mihók, S.: Gidrán lovak kancacsaládjainak ellenőrzése a D-loop régió egy szakaszának vizsgálatával.
In: III. Interdiszciplináris Doktorandusz Konferencia 2014. Konferenciakötet [elektronikus dokumentum]. Szerk.: Schaub Anita, Szabó István, Pécsi Tudományegyetem Doktorandusz Önkormányzat, Pécs, 1061-1066, 2015. ISBN: 9789636427412

Foreign language Hungarian book chapters (1)

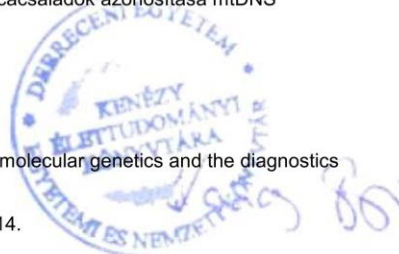
2. **Sziszkosz, N.**, Kusza, S., Jávör, A., Mihók, S.: Determining Gidran mare families using mitochondrial DNA marker.
In: 20th Youth Scientific Forum. Ed.: Bene Szabolcs, University of Pannonia, Georgikon Faculty, Keszthely, 528-537, 2014. ISBN: 9789639639577

Hungarian scientific articles in Hungarian journals (2)

3. **Sziszkosz, N.**, Kusza, S., Jávör, A., Mihók, S.: Méneskönyvi és molekuláris genetikai egybeesések a gidrán kancacsaládjainál az mtDNS alapján.
Agrártud. közl. 65, 69-73, 2015. ISSN: 1587-1282.
4. **Sziszkosz, N.**, Kusza, S., Jávör, A., Mihók, S.: A hucul kancacsaládok azonosítása mtDNS markerrel.
Agrártud. közl. 57, 75-79, 2014. ISSN: 1587-1282.

Foreign language scientific articles in Hungarian journals (1)

5. **Sziszkosz, N.**, Jávör, A., Kusza, S.: Development of horse molecular genetics and the diagnostics of most important monogenic hereditary diseases.
Állatteny. takarm. 65 (3), 71-81, 2016. ISSN: 0230-1814.



Address: 1 Egyetem tér, Debrecen 4032, Hungary Postal address: Pf. 39. Debrecen 4010, Hungary
Tel.: +36 52 410 443 Fax: +36 52 512 900/63847 E-mail: publikaciok@lib.unideb.hu, Web: www.lib.unideb.hu



Foreign language scientific articles in international journals (1)

6. **Sziszkosz, N.**, Mihók, S., Jávör, A., Kusza, S.: Genetic diversity of the Hungarian Gidran horse in two mitochondrial DNA markers.
PeerJ. 4, 1-15, 2016. EISSN: 2167-8359.
DOI: <http://dx.doi.org/10.7717/peerj.1894>
IF: 2.177

List of other publications

Hungarian books (1)

7. Czeglédi, L., **Sziszkosz, N.**, Kusza, S.: Molekuláris biológiai technikák az élelmiszer eredetvizsgálatban. [s.n.], [s.l.], 79 p., 2015.

Foreign language scientific articles in international journals (2)

8. Kusza, S., **Sziszkosz, N.**, Nagy, K., Masala, A., Kukovics, S., Jávör, A.: Preliminary result of a genetic polymorphism of [beta]-lactoglobulin gene and the phylogenetic study of ten balkan and central european indigenous sheep breeds.
Acta Biochim. Pol. 62 (1), 109-112, 2015. ISSN: 0001-527X.
DOI: http://dx.doi.org/10.18388/abp.2014_846
IF: 1.187
9. Tropea, G., Parmakelis, A., **Sziszkosz, N.**, Balanika, K., Boudierka, A.: A New Species of *Euscorpius* Thorell, 1876 from Naples Province, Italy (Scorpiones: Euscorpiidae).
Euscorpius. 182, 1-12, 2014. EISSN: 1536-9307.

Total IF of journals (all publications): 3,364

Total IF of journals (publications related to the dissertation): 2,177

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

28 August, 2017

